Original research

Elevated plasma level of Pentraxin 3 is associated with emphysema and mortality in smokers

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ABSTRACT

Background Pentraxin 3 (PTX3) influences innate immunity and inflammation, host defence, the complement cascade and angiogenesis. PTX3 expression in lung and blood of subjects with tobacco exposure, and its potential relationship with disease pattern and clinical outcome are poorly understood.

Methods Using independent platforms and cohorts, we identified associations of PTX3 gene expression in lung tissue and plasma from current and former tobacco smokers (with and without chronic obstructive pulmonary disease, COPD) to disease phenotypes including quantitative CT determined emphysema, lung function, symptoms and survival. Two putative regulatory variants of the *PTX3* gene were examined for association with COPD manifestations. The relationship between plasma PTX3 and hyaluronic acid levels was further examined.

Results *PTX3* gene expression in lung tissue was directly correlated with emphysema severity (p<0.0001). Circulating levels of PTX3 were inversely correlated with FEV_1 (p=0.006), and positively associated with emphysema severity (p=0.004) and mortality (p=0.008). Two *PTX3* gene regulatory variants were associated with a lower risk for emphysema and expiratory airflow obstruction, and plasma levels of PTX3 and hyaluronic acid were related.

Conclusions These data show strong and overlapping associations of lung and blood PTX3 levels, and *PTX3* regulatory gene variants, with the severity of airflow obstruction, emphysema and mortality among smokers. These findings have potential implications regarding the pathogenesis of smoking-related lung diseases and warrant further exploration for the use of PTX3 as a predictive biomarker.

Key messages

What is the key question?

► We questioned whether Pentraxin 3 (PTX3) expression in lung tissue and blood was associated with clinical lung disease phenotypes in tobacco exposed individuals.

What is the bottom line?

► Lung and blood PTX3 levels, as well as the presence of *PTX3* regulatory gene variants, are associated with the severity of airflow obstruction, emphysema and mortality among smokers.

Why read on?

► The association of PTX3 expression with lung disease phenotypes has potential implications regarding the pathogenesis of smoking-related lung diseases and illuminate the needs for future systematic characterisation of PTX3's role in chronic obstructive pulmonary disease.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the World.[1] COPD manifests with expiratory airflow obstruction due to chronic bronchiolar inflammation and remodelling and or increased lung compliance associated with emphysematous destruction of alveolar septae.[2] COPD and emphysema are most commonly linked to inhalation of noxious substances (eg, tobacco smoke). However, the pathophysiologic processes involved in development and/or progression of COPD/emphysema are complicated and likely multifactorial, including the presence of predisposing genetic elements, aberrant expression of proteases, oxidative stress, mucus dysfunction, infectious agents, abnormal innate and adaptive immune responses, autophagy, cellular senescence and ageing and autoimmunity.[2 3] Several peripheral proteins have demonstrated predictive characteristics in COPD for obstruction and emphysema severity, exacerbation and mortality risk.[4] Only fibrinogen however has achieved qualification by the Food and Drug Administration as a stratifying biomarker for exacerbation and mortality risk.[5]

Pentraxin 3 (PTX3) is a member of the pentraxin superfamily that plays important roles in innate immunity, inflammation and allergy.[6] Serum levels of PTX3 is higher in COPD patients compared with subjects with lung cancer.[7] Abnormal elevations of circulating PTX3 are correlated with the severity of acute lung injury[8] and lung allograft dysfunction.[9] Genetic variations in *PTX3* appear to be determinants of pulmonary microbial infections.[10 11]

We have further examined potential associations between tobacco-associated pulmonary injury and PTX3. These studies include establishing correlations between lung-specific *PTX3* gene expression and plasma levels of PTX3 from emphysema patients with measures of lung disease severity and mortality using data from the Lung Genomic Research Consortium (LGRC)[12] and a well-characterised clinical cohort of current or former

smokers at the University of Pittsburgh. Regulatory variants of the *PTX3* gene were also correlated with spirometric expiratory airflow obstruction and quantitative imaging metrics of emphysema severity. This is the first comprehensive study that examines clinical correlations of PTX3 in large populations of smokers.

METHODS

Study cohorts

Plasma specimens were obtained from sequential participants in the University of Pittsburgh Specialized Center for Clinically Oriented Research (SCCOR) for COPD who completed baseline evaluations and consented to experimental studies using their samples. Complete descriptions of SCCOR subject recruitment and clinical evaluations were previously detailed.[13] In brief, participants were ≥40-years old current or former cigarette smokers with ≥10 pack-year exposures. Each subject completed demographic and medical history questions, as well as evaluations with high resolution chest CT scans, spirometry and measurement of lung diffusion capacity using American Thoracic Society standards and standard reference equations.[13–15] Mortality was assessed by the Social Security Death Index, last queried in August 2018, and subjects' family report.

Explanted lungs from subjects with International Classification of Diseases, Tenth Revision diagnostic codes of COPD and idiopathic pulmonary fibrosis (IPF), who had lung transplant at the University of Pittsburgh Medical Center were obtained from excess pathologic tissues under a protocol approved by the University of Pittsburgh Institutional Review Board (STUDY18100070). Donor lungs not used for transplantation were obtained through the Center for Organ Recovery and Education as controls. Lung tissues were stored at −80°C until future usage. The tissue samples were randomly selected based on diagnosis without any specific exclusion criteria.

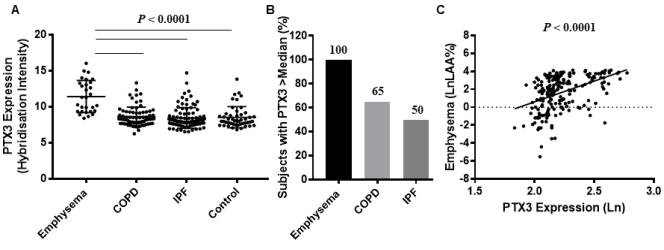

Figure 1 *PTX3* gene expression is upregulated in emphysematous lungs and directly correlated with the degree of emphysema. *PTX3* gene expression levels of 209 COPD, 100 IPF and 48 control lungs of the Lung Genomic Research Consortium cohort were compared. (A) PTX3 gene expression levels from four groups of lung tissues: COPD with severe emphysema (defined as LAA >40%, n=29), and COPD without emphysema (LAA <3%, n=75), IPF (n=100) and controls (n=48). Pair-wise analysis of *PTX3* gene expression in emphysematous lungs of COPD patients compared with those of COPD patients without emphysema (11.4±2.2 vs 8.6±1.3 normalised hybridisation signal, p<0.0001), to IPF patients (8.4±1.4, p<0.0001) or to controls (8.5±1.5, p<0.0001) was performed using the Mann-Whitney test. (B) Percentage of subjects with elevated *PTX3* expression in the lungs using a threshold defined by the median of the *PTX3* levels in the 48 control lungs. (C) Pearson correlation of *PTX3* gene expression with emphysema severity measured by LAA% was performed (R^2=0.19 and p<0.0001) using all COPD subjects with varying degree of emphysema. Both gene expression levels and LAA% were natural log transformed for the correlation analysis. COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; LAA, low-attenuation area; PTX3, Pentraxin 3.

Quantitative and semiquantitative emphysema scoring and airway measurement

Quantitative measurements of emphysema were obtained using low-attenuation area (LAA) defined as the fraction of voxels less than −950 Hounsfield Unit as a percent of total voxels (LAA%) on the CT density histogram.[12 13 16] Semiquantitative measurements of emphysema were determined using a previously validated visual scoring system[13 17] and subjects were grouped into none (0%), trace–mild (1%–25%) and moderate–severe (26%–100%) for the correlation analyses with plasma PTX3 levels and single nucleotide polymorphism (SNP) genotypes. Airway wall thickness, internal perimeter, and wall area of airways were determined based on the chest CT.

Exacerbation index

The acute exacerbation index was derived from questions 5 and 6 of the St. George's Respiratory Questionnaire. Index values range from 0 to 2, with a value of 2 corresponding to the highest acute exacerbation burden (2=response 'More than three attacks' for question 5 and 'A week or more' or '3 days or more' for question 6; 1=response 'Three attacks' or 'Two attacks' for question 5 and 'A week or more' or '3 days or more' for question 6; 0=response 'One attack' or 'No attacks' for question 5 and '1 or 2 days' or 'Less than a day' for question 6).

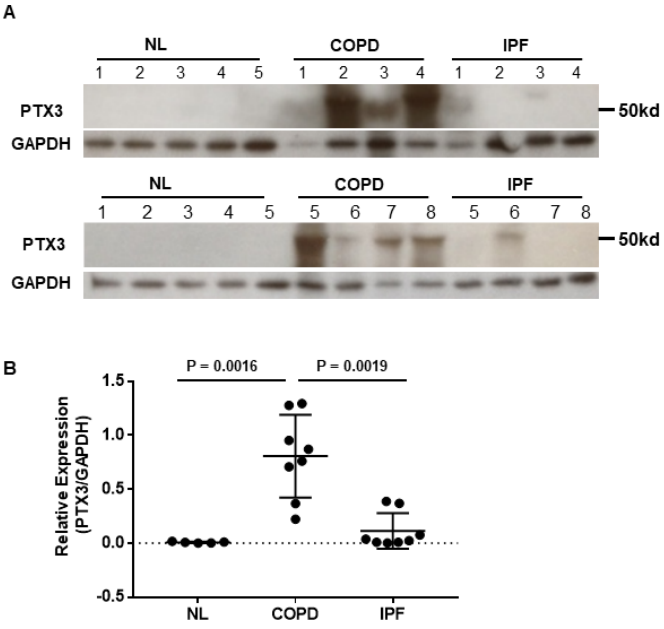

Figure 2 PTX3 protein is upregulated in lungs of advanced COPD. (A) PTX3 protein levels were analysed in lung tissues from subjects with advanced COPD (n=8), IPF (n=8) and controls (n=5) using Western blot with a goat polyclonal anti-PTX3 antibody (R&D Systems, Minneapolis, MN). GAPDH was analysed using a rabbit polyclonal anti-GAPDH (Abcam, Cambridge, MA) as a loading control. (B) Semiquantification of the western blots was performed using Image Lab software (Bio-Rad). Relative expression of PTX3 in the lung tissues was computed based on optical density (density^{PTX3}/density^{GAPDH}). Values are means and SD. Non-parametric Mann-Whitney t-test was used to compare COPD with control (p=0.0016) and COPD with IPF (p=0.0011) lungs. NL, normal lung; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; PTX3, Pentraxin 3.

Table 1 Clinical and demographic characteristics of the Pittsburgh SCCOR tobacco exposed cohort

	N	Median (IQR)*
Age (years)	615	64 (60; 69)
Male	615	317 (51.5)
BMI (kg/m^2)	615	27.5 (24.4; 30.7)
White	615	588 (95.6)
Current smoker	614	220 (35.8)
Pack years of smoking	612	48 (34; 67.5)
FEV_1, % predicted	605	81 (52; 95)
FVC, % predicted	605	91 (81; 101)
FEV_1/FVC	615	65 (44; 75)
DLCO, % predicted	599	67 (47; 83)
LAA%	583	1.3 (0.5; 8.3)
Visual score	608	
No		200 (32.9)
Trace–mild		216 (35.5)
Moderate–severe		192 (31.6)
All airway wall area (%)	586	47.8 (44.5; 51.1)
SGRQ score	614	21.4 (8.4; 46.2)
MMRC score	611	2 (1; 4)
Exacerbation index	614	
0		461 (75.1)
1		78 (12.7)
2		75 (12.2)
Inhaled steroid use	614	178 (28.3)
Oral steroids use	615	51 (8.3)

LAA% less than −950 Hounsfield Unit on a CT scan; visual score, based on a validated six-point visual emphysema score system, with none (0%), trace–mild (1%–25%), moderate–severe (26%–100%) .
*For categorical variables, the actual number and percentage are shown.
BMI, body mass index; DLCO, diffusing capacity of carbon monoxide; LAA%, percentage of low-attenuation area; MMRC score, Modified Medical Research Council Dyspnoea Score; SCCOR, Specialized Center for Clinically Oriented Research; SGRQ, St. George's Respiratory Questionnaire.

Lung tissue-specific *PTX3* gene expression in emphysematous lungs

Lung tissue expressions for the *PTX3* gene were obtained from the LGRC gene expression data set (GEO accession GSE47460; http://www.lung-genomics.org/).[12] Expression levels of *PTX3* from subjects with COPD,[18] IPF and control lungs were used. Direct comparison between COPD specimens with severe emphysema (LAA >40%) and COPD with no emphysema (LAA <3%) was performed. The levels of *PTX3* in COPD with severe emphysema were also compared with that in IPF or controls.

Western blot analysis of PTX3 protein expression in lung tissues

Twenty micrograms of lung parenchyma protein were used in Western blot analysis, employing goat polyclonal anti-PTX3 antibodies (R&D Systems) and rabbit polyclonal anti-GAPDH (glyceraldehyde 3-phosphate dehydrogenase, Abcam). Semi-quantification of the western blots was performed using Image Lab software (Bio-Rad). Relative expression of PTX3 was computed based on optical density ($density^{PTX3}/density^{GAPDH}$).

Plasma level of PTX3 and hyaluronic acid

Plasma samples were collected using citrated blood from SCCOR subjects at their initial visit and banked at −80°C. Plasma levels of PTX3 were analysed using an ELISA duoset according to the manufacturer's instructions (R&D Systems). The range of detection is 218.00–14 000 pg/mL according to the manufacturer. Plasma levels of hyaluronic acid (HA) were analysed using the Hyaluronan Quantikine ELISA Kit (R&D Systems). All samples were analysed in duplicate.

Genotyping

Genomic DNA was isolated from whole blood using either the Puregene or QiaAmp blood DNA isolation kit (QIAgen). SNP rs7616177 and rs2305619 of the *PTX3* gene were genotyped using Taqman method and a 7900 HT DNA analyzer (ABI). For rs7616177, primers 5-GGGCAGGCCCAATTTCAC-3 and 5-GGTAGGAAGCAATCATGTCTCTCTT-3 and probe CAAGGTCCCT[T/C]TGAGTTT were used. A Taqman assay (C__22275654_10, QIAgen) was used for rs2305619 genotyping.

Statistical analysis

For the genetic analysis, we tested allele frequency for departures from Hardy-Weinberg equilibrium using χ^2-testing. In the case–control study, smokers with (LAA ≥3%) or without emphysema (LAA <3%) were used as cases and controls, respectively (prospectively and arbitrarily chosen as a threshold associated with visually normal imaging). Genotype and phenotype correlations were performed using general linear models. Analysis of covariance was used to assess dominant and recessive models. All models were adjusted for age, sex and smoking status.

PTX3 expression levels from lung tissues of COPD patients with severe emphysema were compared with those from tissues of COPD patients without emphysema, controls and IPF patients using the Mann-Whitney test. Correlation of *PTX3* gene expression levels and quantitative emphysema was analysed using linear regression modelling and Pearson correlation. Both *PTX3* gene expression levels and quantitative emphysema were log transformed prior to regression analysis. Lung tissue analysis of PTX3 protein levels with advanced COPD were compared with those with IPF or controls using the Mann-Whitney test. Plasma level of PTX3 were log-transformed and correlated with clinical features using general linear regression analysis and Pearson correlation.

The subjects were divided into equal four groups (quartiles) based on the plasma PTX3 levels and the upper three quartiles were reported as 25th, 50th and 75th percentiles in the results. The association of continuous and categorical variables with PTX3 quartiles was tested using linear test for trend before and after adjusting for age, sex and smoking status. Time to death (or last follow-up) from the first visit was calculated for each study subject. Survival analysis was performed using a Cox proportional hazards model with adjustment for age, sex and smoking status and censoring of patients who underwent lung transplantation. Probability of survival was summarised using Kaplan Meier survival plot. HA levels were compared between subjects with the highest (n=80) and the lowest (n=80) PTX3 levels using a Student's T test. All analyses were performed using Stata V.16.1 (StataCorp), SAS V.9.3 (SAS Institute) or Prism 5 (GraphPad). We used a two-sided test for all hypotheses.

Table 2 Clinical and demographic characteristics by quartiles of plasma PTX3 levels

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value	Adj.P
PTX3 Log (pg/mL)	5.56 (5.35; 5.69)	6.02 (5.90; 6.11)	6.41 (6.31; 6.52)	6.78 (6.95; 7.31)		
Age (years)	62 (59; 67)	65 (60; 70)	65 (61; 70)	66 (62; 71)	**<0.0001**	
Male	78 (51.0)	82 (53.3)	72 (46.8)	85 (55.2)	0.733	
BMI (kg/m^2)	28.7 (25.3; 31.8)	27.8 (25.4; 31.2)	27.7 (24.3; 30.7)	26.5 (23.6; 29)	**<0.0001**	**0.0002**
Current smoker	66 (43.1)	61 (39.6)	45 (29.4)	48 (31.2)	**0.0078**	
FEV_1, % predicted	85 (58; 95)	82 (58; 94.5)	79 (53; 96)	74 (38; 93)	**0.0266**	**0.0061**
FEV_1/FVC	68 (49; 77)	65 (48; 76)	66 (44; 75)	60 (39; 72)	**0.0006**	**0.0023**
DLCO, % predicted	73 (58; 85)	67 (46; 80)	68 (48; 83)	64 (40; 82)	**0.0039**	**0.0024**
Exacerbation index					**0.011**	**0.002**
0	121 (79.1)	118 (76.6)	109 (71.2)	113 (73.4)		
1	23 (15.0)	20 (13.0)	22 (14.4)	13 (8.4)		
2	9 (5.9)	16 (10.4)	22 (14.4)	28 (18.2)		
LAA%	0.8 (0.4; 5.1)	1.3 (0.4; 6.6)	1.3 (0.5; 8)	2.5 (0.55; 15)	**0.0018**	**0.004**
Visual score					**0.0012**	**0.002**
No	58 (38.7)	55 (36.2)	49 (32.2)	38 (24.7)		
Trace–mild	55 (36.7)	54 (35.5)	51 (33.6)	56 (36.4)		
Moderate–severe	37 (24.7)	43 (28.3)	52 (34.2)	60 (39.0)		

For continuous variables, median (IQR) are shown; for categorical variables, the actual number and percentage are shown. LAA% less than −950 Hounsfield Unit on a CT scan; visual score, based on a validated six-point visual emphysema score system, with none (0%), trace–mild (1%–25%), moderate–severe (26%–100%). Adj.P, age, sex and current smoking status adjusted p values. P values of <0.05 are shown in bold.
BMI, body mass index; DLCO, diffusing capacity of carbon monoxide ; LAA%, percentage of low-attenuation area; PTX3, Pentraxin 3.

RESULTS

PTX3 gene expression is increased in emphysematous lungs

To determine whether PTX3 is increased in emphysematous lungs, *PTX3* gene expressions from 209 COPD, 100 IPF and 48 control lungs of the LGRC study were compared (online supplemental table S1). The ages among all groups were comparable. IPF subjects were predominately male (67%) compared with control (39.6%) and COPD (56.5%) groups. The COPD group had the lowest percentage of predicted FEV_1%, while the IPF group had the lowest percentage of predicted FVC%. All controls and IPF subjects had no emphysema observed on CT. A direct comparison of *PTX3* levels in COPD subjects with severe emphysema (defined as LAA >40%, n=29) and without emphysema (LAA <3%, n=75) demonstrated significantly higher levels in the severe emphysema group (11.4±2.2 vs 8.6±1.3 normalised hybridisation signal, p<0.0001). Similarly, *PTX3* expression in the severe emphysematous lungs was significantly higher than that in IPF lungs (8.4±1.4, p<0.0001) and controls (8.5±1.5, p<0.0001, figure 1A). *PTX3* levels in all subjects with severe emphysema were above a threshold defined by the median expression level of *PTX3* in control lungs (figure 1B). In contrast, *PTX3* levels were above this threshold in only 65% of COPD subjects without emphysema and 50% of IPF subjects. Furthermore, *PTX3* levels were directly correlated with emphysema severity (R^2=0.19 and p<0.0001) when all COPD subjects were used in the analysis (figure 1C).

PTX3 protein is elevated in advanced COPD lungs

The levels of PTX3 protein in parenchyma of explanted lungs from subjects with advanced COPD were compared with that of donor (control) and IPF lungs. Significantly higher levels of PTX3 protein were detected in COPD lungs (n=8) compared with both control (n=5, p=0.0016) and IPF (n=8, p=0.0019) lungs (figure 2A,B).

Higher levels of plasma PTX3 are correlated with more impaired lung function and emphysema severity

The relationship between PTX3 and emphysema was further evaluated using plasma levels of PTX3 in subjects from the Pittsburgh SCCOR cohort. A total of 615 ever smokers classified by the Global Initiative for Chronic Obstructive Lung Disease criteria as 0–4 were included in this study (table 1).[18] The median age was 64-years old (range 40–83) and 51.5% were male. The median level of plasma PTX3 was 504.4 pg/mL (IQR: 337.5–741.1). Clinically, 63.9% of subjects had airflow obstruction (<70% FEV_1/FVC) and 38.4% had emphysema quantitatively measured on CT (LAA ≥3%).

To demonstrate the relationships of plasma PTX3 levels with demographic and clinical phenotypes, we divided the cohort into equal quartiles based on PTX3 levels. The demographic and clinical phenotypic characteristics of each quartile are shown in table 2. Participants in the higher quartiles of PTX3 levels were older, had lower BMI and fewer were current smokers. There was no difference in sex across the quartiles. Higher plasma levels of PTX3 were associated with more severe lung function impairment, with differences observed in FEV_1%, FEV_1/FVC and percentage of predicted diffusing capacity of carbon monoxide (DLCO%) across quartiles. The association between PTX3 and DLCO was independent of FEV_1% (p=0.024). These differences remained significant after adjustment for age, sex and current smoking status.

Emphysema severity represented by both quantitative LAA% and semiquantitative visual score was higher in subjects above the 75th percentile of PTX3 plasma levels (p=0.0018 and 0.0012). These associations remained significant after adjustment for age, sex and current smoking status.

Genetic variants of *PTX3* protects smokers from emphysema

We tested the genetic association of two putative regulatory SNPs, rs7616177 (G/A) and rs2305619 (G/A) in the *PTX3* with

Table 3 Genotype association of *PTX3* variants with emphysema

	LAA (≥3%) n=215	LAA (<3%) n=339	OR (95% CI)	P value*
rs7616177				
Genotype†				
GG	78 (36.3)	90 (26.6)	Ref	
GA	96 (44.6)	169 (49.9)	0.62 (0.41 to 0.93)	**0.022**
AA	41 (19.1)	80 (23.6)	0.58 (0.35 to 0.97)	**0.036**
Dominant				
GG	78 (36.3)	90 (26.6)	Ref	
AA+GA	137 (63.7)	249 (73.4)	0.61 (0.41 to 0.89)	**0.011**
Recessive				
GG+GA	174 (80.9)	259 (76.4)		
AA	41 (19.1)	80 (23.6)	1.28 (0.82 to 2.00)	0.27
rs2305619				
Genotype‡				
GG	78 (36.3)	89 (26.2)	Ref	
GA	96 (44.6)	168 (49.6)	0.61 (0.40 to 0.92)	**0.019**
AA	41 (19.1)	82 (24.2)	0.57 (0.34 to 0.94)	**0.028**
Dominant				
GG	78 (36.3)	89 (26.2)	Ref	
AA+GA	317 (63.7)	250 (73.8)	0.60 (0.40 to 0.88)	**0.009**
Recessive				
GG+GA	78 (80.9)	89 (75.8)	Ref	
AA	41 (19.1)	82 (24.2)	1.31 (0.84 to 2.04)	0.23

P values of <0.05 are shown in bold.
*Age, sex and current smoking status adjusted p values. Control: subjects with <3% LAA. Case: subjects with ≥3% LAA.
†Area under curve=0.68, for recessive=0.67.
‡Area under curve=0.69, for recessive=0.67.
BMI, body mass index; LAA, low-attenuation area; PTX3, Pentraxin 3.

emphysema within the SCCOR subjects. To minimise population stratification, only Caucasian subjects (n=554) were included. The genotype distributions of both SNPs met the expectations of Hardy-Weinberg equilibrium (p>0.1). There was an extremely high Linkage disequilibrium (LD) between these two SNPs (r^2 >0.97; D'=1). In controls, the allele frequency for the major G allele of rs7616177 and rs2305619 was 51.5% and 51.0%, respectively. Genetic association with emphysema (LAA ≥3%) and controls (LAA <3%) demonstrated significant associations for both SNPs (table 3). Subjects carrying the minor A allele (AA or GA genotype) for both SNPs were less likely to have emphysema for rs7616177 (OR: 0.61, 95% CI: 0.41 to 0.89) and for rs2305619 (OR: 0.60, 95% CI: 0.40 to 0.88) compared with subjects with the GG genotype. Therefore, the minor A alleles from both SNPs protect smokers from emphysema under a dominant genetic model.

Carriers of *PTX3* protective alleles have less airflow obstruction and emphysema

Consistent with the minor A alleles from both rs7616177 and rs2305619 being protective for emphysema, carriers of the minor allele (AA or GA) for these two SNPs had significantly higher FEV_1% and FEV1/FVC, lower LAA% and lower visual emphysema score (table 4). The carriers of the A allele for each SNP had lower levels of plasma PTX3, approaching statistical significance (p=0.0989 and 0.0788 for rs7616177 and rs2305619, respectively). Therefore, these two *PTX3* SNPs are associated with both airflow obstruction and emphysema.

Higher plasma levels of PTX3 are associated with more frequent acute exacerbation and increased mortality

In the highest quartile of PTX3 plasma levels, 18.2% of subjects had highest acute exacerbation burden compared with only 5.9% subjects in the lowest quartile (table 2). The 615 subjects were followed for a median 9.2 years (IQR: 8.7–10.1). A total of 145 subjects died and 47 subjects underwent lung transplantation. The median plasma level of PTX3 was 468 pg/mL (IQR: 310–683) for subjects who survived versus 600 pg/mL (IQR: 394–927) for subjects who either died or underwent lung transplant. The 5-year survival analysis showed that a statistically significant higher mortality with higher plasma PTX3 levels was detected using the PTX3 plasma level quartiles (p for trend=0.008, figure 3). This trend remained significant after adjustment for age, sex, current smoking status and FEV_1% (p=0.008). Compared with subjects in the 25th percentile of PTX3 plasma levels, subjects in the 75th percentile of PTX3 plasma levels had a 68% higher risk of death adjusted for age, sex, current smoking status and FEV_1% (HR=1.68, 95% CI: 1.08 to 2.61, p=0.020). When adjusted for emphysema (LAA%) in addition to age, sex and current smoke status, the quartile with the highest plasma levels of PTX3 had an even greater risk of death (HR=2.26, 95% CI: 1.44 to 3.56, p<0.0001). Ten-year survival analysis showed similar results (data are not shown).

Plasma levels of hyaluronic acid and PTX3 are related

To explore the cellular mechanisms of PTX3 in COPD, we analysed HA levels in the 80 subjects with the highest and 80 subjects with the lowest plasma levels of PTX3. The HA levels were higher in the highest group (median: 39.9 ng/mL, IQR: 24.3–59.0) compared with the lowest group (median: 25.5 ng/mL, IQR: 18.5–38.5, p=0.0001).

DISCUSSION

This study comprehensively evaluates the association of PTX3 expression with COPD and specifically parenchymal emphysema disease severity and mortality using multiple modalities. We show that *PTX3* gene expression is higher in emphysematous lungs compared with non-emphysematous COPD, IPF and control lungs using the LGRC data set and that the PTX3 protein is upregulated in advanced COPD lungs. Plasma levels of PTX3 are directly correlated with the impairments of spirometry and emphysema severity and are associated with increased mortality in the Pittsburgh SCCOR cohort of current and former smokers with or without COPD. Finally, two SNPs of the *PTX3* gene are protective for emphysema and associated with better pulmonary function. Our results support a mechanistic relationship between tobacco-associated pulmonary emphysema and PTX3.

Airway narrowing and parenchymal emphysema contribute independently to the airflow obstruction in COPD patients,[19] and parenchymal emphysema can occur in the absence of airflow obstruction.[20] Although high plasma levels of PTX3 are correlated with both lung function impairment and emphysema, our findings support that PTX3 is most closely associated with the emphysema phenotype independent of airflow obstruction. Upregulation of *PTX3* gene expression is only detected in emphysematous COPD lungs and not the lungs from subjects with physiologic obstruction but no anatomic emphysema. Furthermore, PTX3 levels correlate with both quantitative and visual emphysema of smokers with and without airflow obstruction.

Table 4 Genotype–phenotype correlation of *PTX3* variants in ever-smokers

	Genotype mean (median IQR)			P value*
rs7616177 (N)	**GG (179)**	**GA (275)**	**AA (129)**	
logPTX3	6.29 (5.87; 6.77)	6.19 (5.76; 6.56)	6.18 (5.86; 6.61)	0.0989
FEV_1, % predicted	76 (38; 94)	82 (57; 95)	82 (50; 98)	**0.0102**
FEV/FVC	60 (39; 72)	66 (48; 76)	67 (47; 77)	**0.0037**
DLCO, % predicted	64 (42; 83)	69 (50; 83)	72 (51; 81)	0.1257
LAA%	2.0 (0.5; 12.7)	1.2 (0.4; 6.6)	0.9 (0.4; 5.3)	**0.0031**
Exacerbation index (%)				0.18
0	72.1	76.5	77.5	
1	13.4	11.6	10.9	
2	14.5	12.0	11.6	
Visual score (%)				**0.030**
No	33.0	33.9	29.7	
Trace–mild	24.4	37.6	46.1	
Moderate–severe	42.6	28.5	24.2	
rs2305619 (N)	**GG (178)**	**GA (275)**	**AA (131)**	
logPTX3	6.30 (5.87; 6.77)	6.19 (5.76; 6.56)	6.18 (5.87; 6.61)	0.0788
FEV_1, % predicted	76 (38; 94)	82 (57; 95)	82 (50; 98)	**0.0106**
FEV/FVC	60 (39; 72)	66 (48; 76)	68 (47; 77)	**0.0033**
DLCO, % predicted	64 (42; 83)	69 (50; 83)	72 (52; 81)	0.1115
LAA%	2.1 (0.5; 12.7)	1.2 (0.5; 6.8)	0.9 (0.4; 5.3)	**0.0027**
Exacerbation index (%)				0.16
0	71.9	76.4	77.9	
1	13.5	11.6	10.7	
2	14.6	12.0	11.5	
Visual score (%)				**0.022**
No	32.6	34.1	30.0	
Trace–mild	24.6	37.4	46.2	
Moderate–severe	42.9	28.6	23.9	

P values of <0.05 are shown in bold.
*Age, sex and current smoking status adjusted p value for dominant model (AA+GA vs GG genotype).
DLCO, diffusing capacity of carbon monoxide; LAA, low-attenuation area; PTX3, Pentraxin 3.

Finally, both the plasma levels and SNP genotypes of PTX3 are more strongly correlated with LAA% than FEV_1% (tables 2 and 4). Our results thus extend the findings from small studies that investigated the relationship between PTX3 and COPD.[7 21 22]

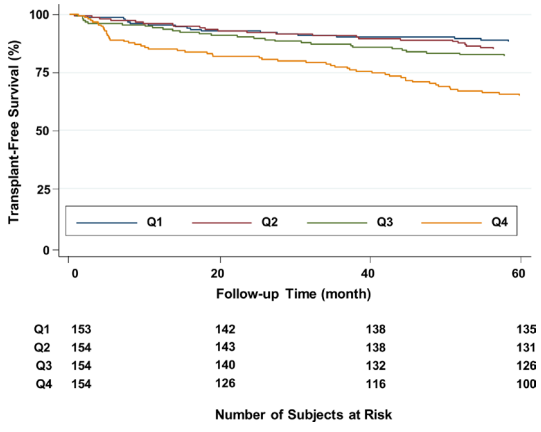

Figure 3 Kaplan-Meier transplant-free survival curves by quartiles of plasma PTX3 levels in smokers with or without COPD. Subjects at risk in each quartile at 0, 20, 40 and 60 months follow-up times are shown, p value for trend=0.008. COPD, chronic obstructive pulmonary disease.

In addition, we have observed an association of higher plasma PTX3 levels with greater acute exacerbation burdens and mortality.

Vascular remodelling is an important feature of emphysema development[23] and PTX3 plays important roles in vascular biology and angiogenesis.[24] Over expression of PTX3 in mouse microvascular endothelial cells led to down-regulation of angiogenesis properties of these cells,[25] and the expression of PTX3 in pulmonary veins is induced by cigarette smoking, the most important risk factor for emphysema.[26] We observe an inverse association of plasma PTX3 levels with DLCO independent of airflow obstruction severity. DLCO has been associated independently with pulmonary hypertension (PH) in COPD.[27] Future mechanistic studies of PTX3 in both emphysema and COPD-associated PH will provide insight into PTX3 function. Other basic mechanisms associated with emphysema include aberrant protease activity and matrix remodelling,[28] abnormal innate immunity and inflammation and dysregulated complement activation.[29 30] Although there is no direct evidence linking PTX3 to these molecular pathways in emphysema, there are many studies demonstrating the importance of this protein in innate immunity[6] and in its interaction with cellular ligands such as components of the complement activation cascade and extra cellular matrix molecules.[31 32] The relationship between plasma

levels of PTX3 and HA suggests that PTX3 may mediate its effects in COPD pathogenesis, in part, through its interaction with matrix HA. Interaction with other molecules in the extra cellular matrix is important for HA's roles in inflammation and cell proliferation.[33–35] Higher levels of HA and HA degrading enzyme hyaluronidase in blood have been associated with severity and mortality in COPD.[36 37] Therefore, future studies dissecting the relationship between PTX3 and HA in COPD pathogenesis are warranted. Finally, the association of plasma PTX3 level with skin elasticity, an emphysema-related phenotype in COPD, suggests a systems biology interaction of PTX3 with markers of multiorgan pathology.[38]

The COPD-associated SNP rs7616177 is localised to 1370 base pairs upstream from the transcription start site and is thus plausibly associated with *PTX3* gene regulation. The rs2305619 SNP is an intronic variant in extremely high LD with rs7616177. Genetic variations of *PTX3* have been associated with infection in stem-cell transplantation recipients and primary graft dysfunction after lung transplantation.[10 39] A protective haplotype is associated with *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis.[11] Recently, variants of *PTX3* were associated with its plasma levels in a population of acute myocardial infarction patients.[40] In our study, the homozygotes of common G alleles, the risk allele for emphysema, for both SNPs trend towards higher plasma PTX3 levels than carriers of the minor A alleles. Reduced lung function is also observed in these homozygotes for both SNPs. However, a recent study showed no correlation between rs2305619 and plasma levels of PTX3 in a COPD cohort.[41] Further comprehensive analysis of genetic variations in the *PTX3* locus is warranted.

Our study does have some limitations. The study lacks a pure replication cohort for both the genetic association and PTX3 levels with emphysema. However, the utilisation of multiple modalities to demonstrate the association between PTX3 and emphysema can be considered an alternate method of validation. Specifically, the PTX3 and emphysema association was first identified based on a cohort of subjects using lung gene expressions and confirmed within a separate cohort using lung tissue proteins from advanced COPD patients. We subsequently confirmed the association of PTX3 levels with emphysema in a smoking cohort of subjects without or with various degrees of airflow obstruction and emphysema. Putative regulatory SNPs of *PTX3* were also associated with reduced predisposition to emphysema and less disease severity. Finally, we provided evidence that PTX3 levels predict mortality in smokers with or without COPD. Although it is beyond the scope of this study, *in vivo* analysis using a mouse model of cigarette smoke induced emphysema and knockout mice would add mechanistic confirmation of a role for PTX3 in the molecular pathogenesis of emphysema.

In conclusion, our study demonstrates associations of pulmonary emphysema with PTX3 expression levels in lung tissues and peripheral blood as well as with genetic variants in smokers with and without airflow obstruction. These findings, including the association of PTX3 with plasma hyaluronan, offer potential mechanistic insights into emphysema pathogenesis. While our findings are consistent across several platforms within different cohorts, further measurements, in existing cohorts as they mature longitudinally, are necessary to determine the potential role of PTX3 as a clinical biomarker.

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Patient consent for publication Not required.

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